

QUANTITATIVE REITER PROTEIN COMPLEMENT-FIXATION TEST*†

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A positive result in the standard serological tests for syphilis (STS) often requires verification by treponemal antibody tests. These tests, however, have their limitations and the results require careful evaluation. The *Treponema pallidum* immobilization (TPI) test is probably the most specific, but it should be kept in mind that it is not positive in every case of syphilis (Kjellander, Sievers, and Vogelsang, 1962; Wuepper, Bodily, and Tuffanelli, 1966). The Reiter protein complement-fixation (RPCF) test is based on a protein antigen common to both *Treponema pallidum* and the Reiter treponeme. It is cheaper and easier to perform than the TPI test and is therefore widely used in the serological diagnosis of syphilis. The RPCF test is usually performed only qualitatively although the use of a quantitative test has been recommended (Bekker, de Bruijn, and Miller, 1966).

The purpose of the present paper is to report the results obtained with a quantitative RPCF test in a series of TPI-tested syphilitic and problem sera.

Material and Methods

A series of 1,665 serum samples sent to the State Serum Institute, Helsinki, for a TPI test was divided into diagnostic groups according to the available clinical information:

(1) *Primary* (112) Symptomatic darkfield-positive cases.

(2) *Secondary* (51) Cases with clinical signs of secondary syphilis and a history of a recent infection, some being darkfield-positive.

(3) *Congenital* (57) Cases with various signs of congenital syphilis, such as parenchymatous keratitis or remnants thereof, Hutchinson's teeth, or skeletal changes combined with positive STS, and also those with sero-positive mother or siblings combined with positive STS.

(4) *Other* (139) This group included both latent and symptomatic cases. The former had some anamnestic evidence and both serological and clinical evidence of syphilis (*e.g.* positive spinal fluid test, aortic aneurysm, or clinical tabes). The latter had been treated at special VD clinics or hospitals or their spouses also were known to be sero-positive.

(5) *Probable* (325) Patients previously treated for syphilis (except those included in Group 4) and those with clinical signs, such as aortic aneurysm, suggestive of old syphilis without information about previous treatment. Cases of probable early syphilis were also included in this group (*e.g.* those with primary syphilis in whom the chancre had not been examined for spirochaetes).

(6) *Problem Cases* (981) No information was available or there was no clinical or anamnestic evidence of syphilis.

Methods

The Reiter protein complement-fixation test was performed with the microtechnique of Fulton and Dumbell (1949) using two units of complement. The TPI test was carried out by the technique of Nelson and Mayer (1949) and Nelson and Diesendruck (1951), with small modifications. The FTA-ABS test was performed according to the provisional technique published in 1965 by the Venereal Diseases Laboratory of the Communicable Diseases Center, Atlanta.

Results

The correlation between the TPI results and the RPCF titres in each diagnostic group is presented in the Table (opposite). The RPCF test is known to become positive earlier in the course of syphilitic infection than the TPI test. This is also apparent from the present primary series in which the RPCF test was positive in 41 and the TPI test in 31 per cent. In all other forms of syphilis our RPCF test was less sensitive than the TPI test. This may be due in part to the circumstance that the lowest serum dilution tested in the RPCF test was one in four.

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TABLE
CORRELATION BETWEEN TPI RESULTS AND
RPCF TITRES IN THE DIFFERENT DIAGNOSTIC
GROUPS

Diagnosis	No. of Cases	TPI Results	RPCF Titre					
			<4	4	8	16	32	≥64
Primary Syphilis	112	Reactive	10	5	8	3	4	5
		Non-reactive	56	6	1	0	1	2
Secondary Syphilis	51	Reactive	9	1	2	0	8	25
		Non-reactive	5	0	1	0	0	0
Congenital Syphilis	57	Reactive	25	12	3	2	0	0
		Non-reactive	14	1	0	0	0	0
Other Syphilis	139	Reactive	39	16	18	15	11	16
		Non-reactive	19	4	0	0	0	1
Probable Syphilis	325	Reactive	68	59	36	18	14	12
		Non-reactive	109	7	1	0	0	1
Problem Cases	981	Reactive	131	84	87	50	15	22
		Non-reactive	559	21	6	6	0	0

Primary The RPCF titres varied greatly both in the TPI-reactive and in the TPI non-reactive groups, some very high titres being found in both.

Secondary About 50 per cent. were TPI-reactive and had very high RPCF titres. On the other hand, about 10 per cent. were non-reactive in both tests. Most of the non-reactive patients had early secondary syphilis.

Congenital The TPI and RPCF tests were reactive in 74 and 32 per cent. respectively, the RPCF titres being lower than in any other group.

Other The TPI and RPCF tests were reactive in 83 and 58 per cent. respectively. Some very high RPCF titres were found in the TPI-reactive group, but in only one of the 24 TPI non-reactive patients. This patient had a history of a recent infection and a healed primary ulcer without clinical signs of secondary syphilis.

Irrespective of the stage of syphilitic infection, more high RPCF titres were seen among the TPI-reactive than among the TPI non-reactive patients.

Probable Fewer TPI and RPCF reactive cases were found in this group than among those with definite syphilis, the distribution of RPCF titres in the TPI-reactive group being very similar to that in the group of "Other Syphilis".

Problem Cases 40 per cent. of the 981 patients were TPI-reactive and 30 per cent. RPCF-reactive. The distribution of the RPCF titres among the TPI-reactive patients followed the pattern of the two previous groups.

There were seventy cases in the present series with a non-reactive TPI test and a reactive RPCF test; 22 (31 per cent.) of these were patients with definite primary or secondary syphilis. Serum was still

available from 33 of the remaining 48 cases, and seventeen of these were positive to the FTA-ABS test.

Discussion

In most of the present cases the TPI test was requested because of positive STS or because of signs suggestive of syphilis. Therefore it is not possible to draw any conclusions concerning the relative sensitivities of the STS and our treponemal tests.

The titres of the antibodies reactive both in lipoidal antigen tests and in the TPI test rise during the primary period of syphilis, and reach their peak values during the secondary phase (Hederstedt and Skog, 1964). The present series seems to show that this also applies to the RPCF test.

One of the main advantages of a quantitative test system lies in the greater ease of comparison of results obtained in different laboratories. The quantitative TPI and FTA-ABS tests are very laborious to perform, and the quantitative RPCF test offers an easier means of tracing the appearance of anti-treponemal antibodies during the course of syphilitic infection and their disappearance after treatment.

The RPCF titres of the TPI-reactive patients varied greatly in all forms of syphilis, and except in primary syphilis, the RPCF titres of the TPI non-reactive cases were usually low. However, there were two cases with a RPCF titre of ≥ 64 and a non-reactive TPI test—one patient with probable secondary syphilis and the other with early latent syphilis. In patients with congenital syphilis the RPCF test was definitely less sensitive than the TPI test.

It has been shown that the RPCF test is rather specific when studied in normal (blood donor) sera (Sequeira, 1962). The question of its specificity in problem sera is an important and difficult one. If a reactive TPI test is considered to be a criterion of specificity, there were in the present series seventy cases with a false positive RPCF test, one-third of which had definite primary or secondary syphilis. Sera from 33 of the remaining patients were tested with the FTA-ABS test which was positive in half of them. This might be taken as an indication that a considerable proportion, possibly the majority, of the patients with a positive RPCF test and a non-reactive TPI test had present or past syphilis.

Summary

A quantitative Reiter protein complement-fixation test was studied in a series of 1,665 syphilitic and problem sera. The RPCF test becomes positive earlier than the TPI test and the titres rise during

the primary phase of syphilis, reaching their peak values during the secondary stage. In late symptomatic and latent syphilis the titres were in general rather low, and in congenital syphilis the RPCF was less sensitive than the TPI test.

In the present series there were seventy cases with a positive RPCF and a negative TPI test, of which 22 had definite primary or early secondary syphilis; 33 of the remainder were subjected to the FTA-ABS test, which was positive in seventeen.

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Un test quantitatif de fixation du complément de la protéine de Reiter

RÉSUMÉ

Un test quantitatif de fixation du complément de la protéine de Reiter a été fait sur une série de 1,665 sérums syphilitiques ou non-résolus. Le test RPCF devient positif plus tôt que le test TPI et les titres augmentent pendant le stage primaire de la syphilis atteignant les valeurs de pointe pendant le stage secondaire. Dans les cas avec symptômes de la syphilis tardive et ceux de la syphilis latente les titres étaient en général plutôt bas et dans les cas de syphilis congénitale le test RPCF était moins sensible que le test TPI.

Dans cette même série, 70 cas avaient donné des résultats positifs au test RPCF et des résultats négatifs au test TPI; de ce nombre 22 montraient des signes cliniques de syphilis primaire ou de syphilis secondaire précoce. 33 cas parmi le reste avaient été soumis au test FTA-ABS, et il y eut 17 résultats positifs.